Please amend Table 1, of page 45, to read:

Table 1: Mean Rejection Scores in Wild-Type Mice After Allogenic Transplant

A23

	Untreated	mCTLA4Ig	anti-B7-1 and B7-2 mAB	$B7.1^{-/-}/B7.2^{-/-}$
Day 14	2.4±0.9	2.6±0.7	1.0±1.4	not done
Day 18/28	2.7±0.7	not done	1.3±0.5	0.8±0.4

REMARKS

Claims 1-5 were previously pending. Claims 1-5 have been amended. A "Version with Markings Showing Changes Made" to the claims is presented in Appendix A. Accordingly, claims 1-5 are pending following entry of this amendment.

Amendments to the claims should in no way be construed as an acquiescence to any of the Examiner's rejections. Amendments to the claims are being made solely to expedite prosecution of the above-identified application. Applicants reserve the option to further prosecute the same or similar claims in the instant or in another patent application.

The specification has been amended to correct minor informalities, errors in spelling and punctuation, as well as for the appropriate annotation to TRADEMARKS. A "Version with Markings Showing Changes Made" to the specification is presented in Appendix A.

No new matter has been added. Support for the amendments to the claims can be found in the application and/or claims as filed or previously pending.

Requested Corrections to the Specification

The Examiner has required that the application be reviewed and all spelling, TRADEMARKS, and like errors be corrected. Applicants have made every effort to detect and correct such errors in the application. Applicants submit that the trademarks

known to Applicants are capitalized, that the proprietary nature of the marks has been respected, and that every effort has been made to prevent their use in any manner which might adversely affect their value as trademarks.

Rejection of Claims Under 35 U.S.C. §102(e)

Claims 1-5 have been rejected under 35 U.S.C. §102(e) as anticipated by the disclosure of Sayegh et al. (US Patent No. 6,280,957). This rejection has been obviated by amendment to claims 1, 3-5.

In making this rejection the Examiner states:

> Sayegh et al. teach methods of transplanting grafts, including intestines ..., with blockers of the CD28-B7 interactions, including anti-B7-1 and/or anti-B7-2 antibodies ... and immunosuppressive agents capable of inactivating T cells, including rapamycin ... The claimed functional limitations would be inherent properties of the referenced methods to transplant intestines with combination therapies, including the use of anti-B7-1 and anti-B7-2 antibodies and rapamycin.

The claims as amended are directed to methods of downmodulating the immune response to an intestinal allograft in a subject comprising administering a therapeutic composition to the subject wherein the therapeutic composition consists of an antibody that binds to B7-1 and an antibody that binds to B7-2, such that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition.

The claims as amended are further directed to methods of downmodulating the immune response to an intestinal allograft in a subject comprising pretreating said subject prior to said intestinal allograft with a therapeutic composition consisting of an antibody that binds B7-1, an antibody that binds B7-2 and a rapamycin compound, such that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition.

The claims as amended are further directed to methods of downmodulating the immune response to an intestinal allograft in a subject comprising post-treating said subject after said intestinal allograft with a therapeutic composition consisting of an antibody that binds B7-1, an antibody that binds B7-2, and a rapamycin compound, such that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition.

The claims as amended are also directed to methods of downmodulating the immune response to an intestinal allograft in a subject comprising pretreating said subject prior to said intestinal allograft and post-treating said subject after said intestinal allograft with a therapeutic composition consisting of an antibody that binds B7-1, an antibody that binds B7-2, and a rapamycin compound, such that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition.

The rejection of claims 1-5 has been obviated by amendment to claims 1 and 3-5, to indicate that the therapeutic composition consists of an antibody that binds to B7-1 and an antibody that binds to B7-2 and that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition. Sayegh et al. teach administration of an antibody that binds B7-1 and an antibody that binds B7-2 with rapamycin, ***in combination with intravenous injection of donor hematopoietic stem cells and, optionally, an inhibitor of CD40:CD40L interaction***. Sayegh et al. neither teaches nor suggests administration of an antibody that binds B7-1 and an antibody that binds B7-2 to a subject in the absence of donor hematopoietic stem cells. In fact, Sayegh et al. teach that administration of CTLA4-Ig alone provides no protection from allograft rejection in their mouse model (Figure 1, discussed in Example 1, column 11). Similar results are also disclosed for skin graft rejection (Figure 2, and columns 11 and 12). These results actually teach away from Applicants invention.

In view of the foregoing, it is respectfully requested that the rejection of claims as anticipated by Sayegh et al. be reconsidered and withdrawn.

Rejection of Claims Under 35 U.S.C. §103(a)

Claims 1-5 have been rejected under 35 U.S.C. §103(a) as being obvious over the disclosures de Boer et al. (U.S. Patent No. 5,869,050) in view of Lenschow et al. (*Transplantation 60:* 1171-1178 (1995)), Tarumi et al. (*Transplantation 67:* 520-525 (1999) and/or Newell et al. (*J Immunol. 163:* 2358-2362 (1999). More specifically, the Examiner states:

> Given the teachings of de Boer et al., Lenschow et al., Tarumi et al., and Newell et al., the ordinary artisan would have an expectation of success in prolonging intestinal graft survival by blocking both B7-1 and B7-2-mediated interactions. From the teachings of the references, it was apparent that one of ordinary skill in

the art would have had a reasonable expectation of success in producing the claimed invention.

This rejection is respectfully traversed.

To establish a *prima facie* case of obviousness for the claimed invention, there must have been some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings in the manner proposed by the Examiner. Second, there must have been a reasonable expectation of success at the time the invention was made. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. See M.P.E.P. 2143. The prior art must suggest "to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process" and "[b]oth the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." *In re Dow Chemical Co.* 837 F.2d 469. 473, 5 U.S.P.Q.2d 1529, 1531 (Fed.Cir. 1988).

The pending claims are set forth above.

Applicants argue that, at the time the invention was made, there was no motivation to combine the art in the manner suggested by the Examiner to arrive at the claimed invention. More specifically one of ordinary skill in the art would not have been motivated to modify the teachings of de Boer et al., (which only generally refers to transplant rejection) and the teachings of Lenschow et al. (which discloses studies of inhibition of pancreatic islet transplants) with the teachings of Tarumi et al. or Newell et al., which, although they pertain to intestinal allograft transplantation, teach the use of CTLA4 Ig.

First, prior to the instant disclosure, one of ordinary skill in the art would not have been motivated to specifically target signaling by B7-1 and B7-2 to prolong intestinal allograft transplants. This is, in part, because the nature of the immune response to intestinal allografts seems to be unique compared to other types of allografts and, thus, results obtained from experiments with other types of allografts cannot be extrapolated to transplantation of intestinal grafts. As evidence of this, Applicants refer the Examiner to the disclosure of Yin et al. ((*Transplantation 62:* 1537 (1996)) enclosed as Appendix B) which teaches that therapies which are effective at preventing transplant rejection of

allografts of other types of tissues are ineffective in the prevention of small bowel allograft rejection (page 1537, column 2, lines 1-6 of the first full paragraph).

Second, the art cited by the Examiner fails to provide the necessary motivation to make the claimed invention. Tarumi et al. and Newell et al. are cited as teaching that inhibition of the CD28/CTLA4 pathway in a subject might result in prolonged intestinal allograft survival. The disclosure of Newell et al. does not support the position of the Examiner since it teaches that administration of CTLA4-Ig ***does not*** block intestinal allograft rejection in wild type mice (page 2358, column 2, first paragraph, line 21-23, also page 2359, column 2, first paragraph to page 2360, column 1, first paragraph, and also figure 1). In actuality, inhibition of transplant rejection was reported by Newell et al. to occur only as a result of administration of CTLA4-Ig to CD8 knock out mice (page 2358, column 2, first paragraph, line 21-23, and page 2360, column 2, second paragraph). Therefore, since CTLA4-Ig did not work to prolong intestinal allograft acceptance in normal mice in this reference, one of ordinary skill in the art would not have been motivated to try prolonging intestinal allograft acceptance using antibodies to B7-1 and B7-2.

This teaching of the Newell paper that CTLA4Ig was ineffective in prolonging intestinal allograft transplants was corroborated by the teachings of Yin et al. As discussed above, Yin taught that the inhibition of the CD28/CTLA4 pathway with CTLA4-Ig was insufficient to inhibit transplant rejection of small bowel allografts in their model system. Furthermore, as discussed above, the disclosure of Sayegh et al. teaches that inhibition of CD28/CTLA4 pathway with CTLA4-Ig was insufficient to inhibit transplant rejection.

The Tarumi reference relied upon by the Examiner is the only cited reference that teaches an effect of CTLA4Ig on intestinal allograft survival. In addressing the discrepancy between their data and those of Yin, Tarumi et al. teach that "[w]e used relative a low-responder Lewis/Brown-Norway combination; however, Yin et al. used the high-responder Lewis/ACI combination." Thus, Tarumi et al. confirm that in rats that are not low responders, the CTLA4Ig treatment will not work.

In addition, prior to Applicants demonstration that B7-1 and B7-2 expression by recipient cells was required for transplant rejection using knock out animals, there was a

distinct possibility that CTLA4Ig could have worked (in those situations where it had been shown to work) by binding to an additional costimulatory molecule. Applicants findings were significant in that they indicated that specific targeted inhibition of B7-1 and B7-2 (e.g., via administration of blocking antibodies to these two molecules) were sufficient to reduce the immune response to an intestinal allograft.

Therefore, at the time the invention was made, one of ordinary skill in the art would not have been motivated to modify the teachings of the references and administer a therapeutic composition to the subject wherein the therapeutic composition consists of an antibody that binds to B7-1 and an antibody that binds to B7-2, such that the immune response to an intestinal allograft in a subject is downmodulated.

Further, Applicants argue that, even if the motivation existed to combine the cited references to produce the claimed methods at the time of the invention (which Applicants deny), the ordinarily skilled artisan did not have a reasonable expectation of success at making the claimed invention.

Again, Applicants argue that the rejection of intestinal type allografts is a process which is biologically distinct from the rejection of other tissue and organ grafts, as evidenced by the disclosure of Yin et al., discussed above. At the time of the invention, the ordinarily skilled artisan was aware of this distinction and recognized that teachings in the prior art which were not specific to intestinal allografts, such as the teachings of de Boer et al., and the teachings of Lenschow et al., were insufficient to provide a reasonable expectation of success when applied to the prevention of intestinal allograft rejection.

One or ordinary skill in the art would also have recognized that the remaining disclosures cited by the Examiner (Tarumi et al. and Newell et al.,) which specifically relate to intestinal type allografts, did not compensate for the deficiencies of the disclosures of de Boer et al. and Lenschow et al. The Newell disclosure teaches that administration of CTLA4-Ig is insufficient to block intestinal allograft rejection in wild type mice, as discussed above. Tarumi et al. teach that this therapy does not work in mice with normal responsiveness. Since CTLA4-Ig is known to block B7-1 and B7-2 induced costimulation, this suggests that blockage of B7-1 and B7-2 is insufficient to prevent intestinal allograft rejection.

Although the Tarumi disclosure presents evidence that CTLA4-Ig can prevent intestinal allograft rejection in one model system, this is not corroborated by the prior art, given the disclosure of Newell et al. and also Yin et al., and Sayegh et al., discussed above, each of which were known to the ordinary skilled artisan art at the time of the present invention. Thus, at the time the invention was made, there were known teachings in the art which were contradictory to the teachings of Tarumi et al. and to the position of the Examiner. This controversy in the art at the time of the invention indicates that one or ordinary skill in the art would not have had a reasonable expectation of success at making the claimed invention.

Furthermore, even in the presence of teachings in the art which showed that administration of CTLA4-Ig was sufficient to inhibit intestinal allograft rejection in a low responder mouse model, the ordinarily skilled artisan would not reasonably have expected that specifically targeting B7-1 and B7-2 mediated signaling, e.g., by utilizing blocking antibodies, would produce the same results as use of the CTLA4-Ig molecule, prior to the immediate disclosure. This is true because CTLA4 binds multiple ligands and CTLA4-Ig therefore blocks a wider range of signaling molecules than B7-1 and B7-2 specific antibodies. Applicants' invention is based on a specific analysis of the involvement of B7-1 and B7-2 in intestinal allograft transplant rejection, made in a highly relevant mouse model system. Applicants' findings conclusively indicate that B7-1 and B7-2 expression by recipient cells is required for transplant rejection, since mice that were deficient in B7-1 and B7-2 exhibited significantly reduced rejection. These findings were significant in that they indicated that blockage of B7-1 and B7-2 signaling was sufficient to inhibit transplant rejection in a highly relevant model system. Prior to these findings, there was no indication that blockage of B7-1 and B7-2 signaling was sufficient, given that the CTLA4-Ig molecule was not a specific inhibitor of B7-1 and B7-2 (e.g., it was also taught to inhibit alternative constimulatory receptors which bind CTLA-4 (e.g., B7-3 (Boussiotis et al., *PNAS USA 90:* 11059-63 (1993)). Applicants' determination of the requirement for B7-1 and B7-2 in intestinal type allograft transplant rejection, coupled with exemplification of allograft survival from administration of anti-B7-1 and anti-B7-2 antibodies, ***conclusively*** demonstrated the therapeutic efficacy of the claimed methods.

In light of the above, Applicants maintain that the Examiner has not demonstrated that the combined disclosures of de Boer et al., Lenschow et al., Tarumi et al., and/or Newell et al., provided a reasonable expectation of success to the ordinary skilled artisan at the time the invention was made.

In light of the foregoing, Applicants maintain that the claimed invention is not obvious in view of the teachings of de Boer et al., Lenschow et al., Tarumi et al., and/or Newell et al. and ask that the above rejection be reconsidered and withdrawn.

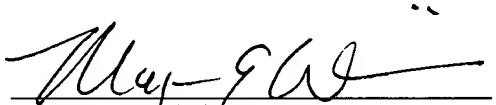
SUMMARY

In view of the above amendments and remarks, Applicants respectfully request reconsideration of the claims and withdrawal of the outstanding rejections.

If a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' attorney at (617) 227-7400.

Respectfully submitted,

LAHIVE & COCKFIELD, LLP



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APPENDIX A

Version With Markings Showing Changes Made to Amended Claims:

1. (Amended) A method of downmodulating the immune response to an intestinal allograft in a subject comprising administering a therapeutic composition to the subject wherein the therapeutic composition consists of an antibody that binds to B7-1 and an antibody that binds to B7-2, such that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition.

2. (Amended) The method of claim 1, [further comprising administering] wherein a second therapeutic composition consisting of a rapamycin compound is administered to the subject.

3. (Amended) A method of downmodulating the immune response to an intestinal allograft in a subject comprising pretreating said subject prior to said intestinal allograft with a therapeutic composition consisting of an antibody that binds B7-1 [B7.1], an antibody that binds B7-2 [B7.2] and a rapamycin compound, such that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition.

4. (Amended) A method of downmodulating the immune response to an intestinal allograft in a subject comprising post-treating said subject after said intestinal allograft with a therapeutic composition consisting of an antibody that binds B7-1 [B7.1], an antibody that binds B7-2 [B7.2], and a rapamycin compound, such that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition.

5. (Amended) method of downmodulating the immune response to an intestinal allograft in a subject comprising pretreating said subject prior to said intestinal allograft and post-treating said subject after said intestinal allograft with a therapeutic composition

consisting of an antibody that binds B7-1 [B7.1], an antibody that binds B7-2 [B7.2], and a rapamycin compound, such that the immune response to an intestinal allograft in a subject is downmodulated by the therapeutic composition.

Version With Markings Showing Changes Made to the Specification:

Page 1, the paragraph under the heading "Related Applications":

The present application claims priority to U.S. Provisional Patent Application Serial No. 60/189,165, filed March 14, 2000, entitled "Use of a Combination of Anti-B7-1 and Anti-B7-2 Antibodies in Inhibiting Intestinal Allo Graft Rejection", the entire contents of which are expressly incorporated herein by reference.

Page 1, the last paragraph:

The CD80 (B7-1) and CD86 (B7-2) [(B7)] proteins, expressed on APCs, are critical costimulatory molecules (Freeman et al. 1991. *J. Exp. Med.* 174:625; Freeman et al. 1989 *J. Immunol.* 143:2714; Azuma et al. 1993 *Nature* 366:76; Freeman et al. 1993. *Science* 262:909). B7-2 [B7] appears to play a predominant role during primary immune responses, while B7-1, which is upregulated later in the course of an immune response, may be important in prolonging primary T cell responses or costimulating secondary T cell responses (Bluestone. 1995. *Immunity.* 2:555).

Page 2, the first paragraph:

One receptor to which B7-1 and B7-2 [B7] bind, CD28, is constitutively expressed on resting T cells and increases in expression after activation. After signaling through the T cell receptor, ligation of CD28 and transduction of a costimulatory signal induces T cells to proliferate and secrete IL-2 (Linsley, P.S., et al. 1991 *J. Exp. Med.* 173, 721-730; Gimmi, C.D., et al. 1991 *Proc. Natl. Acad. Sci. USA.* 88, 6575-6579; June,

C.H., et al. 1990 *Immunol. Today.* 11, 211-6; Harding, F.A., et al. 1992 *Nature.* 356, 607-609). A second receptor, termed CTLA4 (CD152) is homologous to CD28 but is not expressed on resting T cells and appears following T cell activation (Brunet, J.F., et al., 1987 *Nature* 328, 267-270). CTLA4 appears to be critical in negative regulation of T cell responses (Waterhouse et al. 1995. *Science* 270:985). Blockade of CTLA4 has been found to remove inhibitory signals, while aggregation of CTLA4 has been found to provide inhibitory signals that downregulate T cell responses (Allison and Krummel. 1995. *Science* 270:932). The B7 molecules have a higher affinity for CTLA4 than for CD28 (Linsley, P.S., et al., 1991 *J. Exp. Med.* 174, 561-569) and B7-1 and B7-2 [B7] have been found to bind to distinct regions of the CTLA4 molecule and have different kinetics of binding to CTLA4 (Linsley et al. 1994. *Immunity.* 1:793).

Page 1, the last paragraph:

The importance of the B7:CD28/CTLA4 costimulatory pathway has been demonstrated *in vitro* and in several *in vivo* model systems. Blockade of this costimulatory pathway results in the development of antigen specific tolerance in murine and humans systems (Harding, F.A., et al. (1992) *Nature.* 356, 607-609; Lenschow, D.J., et al. (1992) *Science.* 257, 789-792; Turka, L.A., et al. (1992) *Proc. Natl. Acad. Sci. USA.* 89, 11102-11105; Gimmi, C.D., et al. (1993) *Proc. Natl. Acad. Sci USA* 90, 6586-6590; Boussiotis, V., et al. (1993) *J. Exp. Med.* 178, 1753-1763). Conversely, expression of B7-1 [B7] by B7-1 [B7] negative murine tumor cells induces T-cell mediated specific immunity accompanied by tumor

Page 3, paragraphs 4-6:

In another embodiment, the invention provides a method of downmodulating the immune response to an intestinal allograft in a subject comprising pretreating the subject prior to the intestinal allograft with an antibody that binds to B7-1 [B7.1], an antibody that binds to B7-2 [B7.2], and a rapamycin compound.

In another embodiment, the invention provides a method of downmodulating the immune response to an intestinal allograft in a subject comprising post-treating the subject after the intestinal allograft with an antibody that binds to subject with an antibody that binds to [B7.] B7-1, an antibody that binds to B7-2 and a rapamycin compound.

In another embodiment, the invention provides a method of downmodulating the immune response to an intestinal allograft in a subject comprising pretreating the subject before the intestinal allograft and post-treating the subject after the intestinal allograft with an antibody that binds to B7-1 [B7.1], an antibody that binds to B7-2 [B7.2], and a rapamycin compound.

Page 4, the fourth paragraph:

As used herein, the term "costimulate" with reference to activated immune cells includes the ability of a costimulatory molecule to provide a second, non- activating receptor mediated signal (a "costimulatory signal") that induces proliferation or effector function. For example, a costimulatory signal can result in cytokine secretion, e.g., in a T cell that has received a T cell-receptor-mediated signal. As used herein the term "costimulatory molecule" includes molecules which are present on antigen presenting cells (e.g., B7-1, B7-2 [B7], B7RP-1 (Yoshinaga et al. 1999. Nature 402:827), B7h (Swallow et al. 1999. Immunity. 11:423) and/or related molecules (e.g., homologs)) that bind to costimulatory receptors (e.g., CD28, CTLA4, ICOS (Hutloff et al. 1999. Nature 397:263), B7h ligand (Swallow et al. 1999. Immunity. 11:423) and/or related molecules) on T cells. These molecules are also collectively referred to herein as "B7 molecules."

Page 7, the last paragraph:

As used herein, the term "extracellular domain of a B7 molecule" includes a portion of a B7 molecule which, in the cell-associated form of a B7 molecule, is extracellular. A B7 extracellular domain includes the portion of a B7 molecule which mediates binding to a costimulatory receptor, e.g., CD28, ICOS, and/or CTLA4. For

example, the human B7-1 extracellular domain comprises from about amino acid 1 to about amino acid 208 and the human B7-2 [B7] extracellular domain comprises from about amino acid 24 to about amino acid 245. See, for example, B7-2 (Freeman et al. 1993 *Science*. 262:909; GenBank Accession numbers P42081 or A48754; or United States Patent 5,942,607); B7-1 (Freeman et al. *J. Exp. Med.* 1991. 174:625; GenBank Accession numbers P33681 or A45803; or United States Patent 5,858,776).

Page 10 the third and fourth paragraphs:

[Purification techniques for B7 molecules have been established, and, additionally, B7 genes (cDNA) have been cloned from a number of species, including human and mouse (see, for example, Freeman, G.J. et al. (1993) *Science* 262:909-911; Azuma, M. et al. (1993) *Nature* 366:76-79; Freeman, G.J. et al. (1993) *J. Exp. Med.* 178:2185-2192).

Nucleotide sequences of costimulatory molecules are known in the art and can be found in the literature or on a database such as GenBank. See, for example, B7-2 (Freeman et al. 1993 *Science*. 262:909 or GenBank Accession numbers P42081 or A48754); B7-1 (Freeman et al. *J. Exp. Med.* 1991. 174:625 or GenBank Accession numbers P33681 or A45803; CTLA4 (See e.g., Ginsberg et al. 1985. *Science*. 228:1401; or GenBank Accession numbers P16410 or 291929); and CD28 (Aruffo and Seed. *Proc Natl. Acad. Sci.* 84:8573 or GenBank Accession number 180091), ICOS (Hutloff et al. 1999. *Nature.* 397:263; WO 98/38216), and related sequences.]

Page 11, the first paragraph:

non-costimulatory molecule genes, (e.g., under conditions equivalent to 65°C in 5 X SSC (1 X SSC = 150 mM NaCl/ 0.15 M Na citrate)) can be used to make anti-B7 [antiB7] antibodies. Alternatively, DNA sequences which retain sequence identity over regions of the nucleic acid molecule which encode protein domains which are important in costimulatory molecule function, e.g., binding to other costimulatory [costimultory] molecules, can be used to produce costimulatory proteins which can be used as

immunogens. Preferably, nonnaturally occurring costimulatory molecules have significant (e.g., greater than 70%, preferably greater than 80%, and more preferably greater than 90-95%) amino acid identity with a naturally occurring amino acid sequence of a costimulatory molecule extracellular domain.

Page 12, the first paragraph:

Using B7 cDNA molecules, peptides having an activity of B7 can be produced using standard techniques. Host cells transfected to express peptides can be any prokaryotic or eukaryotic cell. For example, a peptide having B7 activity can be expressed in bacterial cells such as *E. coli,* insect cells (baculovirus), yeast, or mammalian cells such as Chinese Hamster ovary cells (CHO) and NS0 cells. Other suitable host cells and expression vectors may be found in Goeddel, (1990) *supra* or are known to those skilled in the art. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari. et al., (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series (Smith et al., (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow, V.A., and Summers, M.D., (1989) *Virology* 170:31-39). Generally, COS cells (Gluzman, Y., (1981) *Cell* 23:175-182) are used in conjunction with such vectors as pCDM8 (Seed, B., (1987) *Nature* 329:840) for transient amplification/expression in mammalian cells, while CHO (dhfr$^-$ Chinese Hamster Ovary) cells are used with vectors such as pMT2PC (Kaufman et al. (1987), *EMBO J.* 6:187-195) for stable amplification/expression in mammalian cells. A preferred cell line for production of recombinant protein is the NS0 myeloma cell line available from the ECACC (catalog #85110503) and described in Galfre, G. and Milstein, C. ((1981) *Methods in Enzymology* 73(13):3-46; and *Preparation of Monoclonal Antibodies: Strategies and Procedures*, Academic Press, N.Y., N.Y). Vector DNA can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, LIPOFECTIN™ [lipofectin], or electroporation. Suitable methods for

transforming host cells can be found in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks. When used in mammalian cells, the expression vector's control functions are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and most frequently, Simian Virus 40.

Page 13, the last paragraph:

In one embodiment, variants of a B7 polypeptide which function as either B7 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a B7 (or B7 ligand) polypeptide for B7 antagonist activity. In one embodiment, a variegated library of B7 variants [B7variants] is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of B7 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into

Page 14, the first paragraph:

gene sequences such that a degenerate set of potential B7 or B7 ligand sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of B7 or B7 ligand sequences therein. There are a variety of methods which can be used to produce libraries of potential B7 or B7 ligand variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential B7 or B7 ligand sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S. A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

Page 15, the second and third paragraphs:

In one embodiment, cell based assays can be exploited to analyze a variegated B7 or B7 ligand library. For example, a library of expression vectors can be transfected into a cell line which ordinarily synthesizes B7 or B7 ligand. The transfected cells are then cultured such that B7 or B7 ligand and a particular mutant B7 or B7 ligand are secreted and the effect of expression of the mutant on B7 or B7 ligand activity can be detected, *e.g.*, by any of a number of functional assays. DNA can then be recovered from the cells which score for inhibition of B7 or [B7or] B7 ligand activity, and the individual clones further characterized.

In addition to B7 or B7 ligand polypeptides consisting only of naturally-occurring amino acids, B7 or B7 ligand peptidomimetics are also provided. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds [compound] are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. (1986) *Adv. Drug Res.* 15:29; Veber and Freidinger (1985) TINS p.392; and Evans *et al.* (1987) *J. Med. Chem.* 30:1229, which are incorporated herein by reference) and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides can be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.*, a polypeptide that has a biological or pharmacological activity), such as human B7 or B7 ligand, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -CH2NH-, -CH2S-, -CH2-CH2-, -CH=CH- (cis and trans), -COCH2-, -CH(OH)CH2-, and -CH2SO-, by methods known in the art and further described in the following references: Spatola, A. F. in "*Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*" Weinstein, B., ed., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Morley, J. S. (1980) *Trends Pharm. Sci.* pp. 463-468 (general review); Hudson, D. *et al.* (1979) *Int. J. Pept. Prot. Res.* 14:177-185 (-CH2NH-, CH2CH2-); Spatola, A. F. *et al.*

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(1986) *Life Sci.* 38:1243-1249 (-CH2-S); Hann, M. M. (1982) *J. Chem. Soc. Perkin Trans. I.* 307-314 (-CH-CH-, cis and trans); Almquist, R. G. *et al.* (190) *J. Med. Chem.* 23:1392-1398 (-COCH2-); Jennings-White, C. *et al.* (1982) *Tetrahedron Lett.* 23:2533 (-COCH2-); Szelke, M. *et al.* European Appln. EP 45665 (1982) CA: 97:39405 (1982)(-CH(OH)CH2-);

Page 16, the first and second paragraphs:

Holladay, M. W. *et al.* (1983) *Tetrahedron Lett.* (1983) 24:4401-4404 (-C(OH)CH2-); and Hruby, V. J. (1982) *Life Sci.* (1982) 31:189-199 (-CH2-S-); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is -CH2NH-. Such peptide mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (*e.g.*, a broad-spectrum of biological activities), reduced antigenicity, and others. Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (*e.g.*, an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with the macromolecule(s) [macromolecules(s)] to which the peptidomimetic binds, to produce the therapeutic effect. Derivitization (*e.g.*, labeling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic.

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Systematic substitution of one or more amino acids of a B7 or B7 ligand [ligands] amino acid sequence with a D-amino acid of the same type (*e.g.*, D-lysine in place of L-lysine) can be used to generate more stable peptides. In addition, constrained peptides comprising a B7 or B7 ligand amino acid sequence or a substantially identical sequence variation can be generated by methods known in the art (Rizo and Gierasch (1992) *Annu. Rev. Biochem.* 61:387, incorporated herein by

reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

The amino acid sequences of B7 or B7 ligand polypeptides identified herein will enable those of skill in the art to produce polypeptides corresponding to B7 or B7 ligand peptide sequences and sequence variants thereof. Such polypeptides can be produced in prokaryotic or eukaryotic host cells by expression of polynucleotides encoding a B7 or B7 ligand peptide sequence, frequently as part of a larger polypeptide. Alternatively, such peptides can be synthesized by chemical methods. Methods for expression of heterologous proteins in recombinant hosts, chemical synthesis of polypeptides, and *in vitro* translation are well known in the art and are described further in Maniatis *et al.* *Molecular Cloning: A Laboratory Manual* (1989), 2nd Ed., Cold Spring Harbor, N.Y.; Berger and Kimmel, Methods in Enzymology, Volume 152, Guide to Molecular Cloning Techniques (1987), Academic Press, Inc., San Diego, Calif.; Merrifield, J. (1969) *J. Am. Chem. Soc.* 91:501; Chaiken I. M. (1981) *CRC Crit. Rev. Biochem.* 11: 255; Kaiser *et al.* (1989) *Science*

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Page 17, the first paragraph:

243:187; Merrifield, B. (1986) *Science* 232:342; Kent, S. B. H. (1988) *Annu. Rev. Biochem.* 57:957; and Offord, R. E. (1980) *Semisynthetic Proteins*, Wiley Publishing, which are incorporated herein by reference[])]

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Page 18, the first paragraph:

Moreover, it will be appreciated by those skilled in the art that it is within their skill to generate additional agents and screen for their activity by following standard techniques. For instance, B7 molecules from a variety of species, whether in soluble form or membrane bound, can be used to induce the formation of anti-B7 antibodies. Such antibodies may either be polyclonal or monoclonal, or antigen binding fragments of such antibodies. Of particular significance for use in therapeutic applications are antibodies that inhibit binding of a B7 molecule with its natural ligand(s) on the surface of immune

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cells, thereby inhibiting costimulation of the immune cell through the B7-ligand interaction. Preferred anti-B7 antibodies are those capable of inhibiting or downregulating T cell mediated immune responses by binding the B7 molecule on the surface of B lymphocytes and preventing interaction of the B7 molecule with CTLA4 and/or CD28. Preferably, the combination of antibodies chosen for use in the invention results in increased inhibition of costimulation of an immune cell, such as a T cell, through the B7-ligand interaction, relative to either antibody alone.

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Page 21, the third paragraph:

Such mammal-produced populations of antibody molecules are referred to as "polyclonal" because the population comprises antibodies with differing immunospecificities and affinities for a costimulatory molecule. The antibody molecules are then collected from the mammal and isolated by well known techniques such as, for example, by using DEAE SEPHADEX™ [Sephadex] to obtain the IgG fraction. To enhance the specificity of the antibody, the antibodies may be purified by immunoaffinity chromatography using solid phase-affixed immunogen. The antibody is contacted with the solid phase-affixed immunogen for a period of time sufficient for the immunogen to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

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Page 23, the first paragraph:

(b) A suspension of antibody-producing cells removed from each immunized mammal secreting the desired antibody is then prepared. After a sufficient time, the mouse is sacrificed and somatic antibody-producing lymphocytes are obtained. Antibody-producing cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals. Spleen cells are preferred, and can be mechanically separated into individual cells in a physiologically tolerable medium using methods well known in the art. Mouse lymphocytes give a higher percentage of stable fusions with the mouse myelomas described below. Rat, rabbit and frog somatic cells can also be used. The

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spleen cell chromosomes encoding desired immunoglobulins are immortalized by fusing the spleen cells with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol (PEG). Any of a number of myeloma cell lines may be used as a fusion partner according to standard techniques; for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from the American Type Culture Collection (ATCC™), 10801 University Bou`,levard, Manassas, VA 20110-22009. [Rockville, Md.]

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Page 31, the first paragraph:

chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and Maniatis (eds), *Molecular Cloning; A Laboratory Manual, Second Edition*, Cold Spring Harbor, N.Y., (1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 by Boss *et al.*

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Page 31, the last paragraph:

To express the antibodies, or antigen binding portions of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be

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inserted into separate vectors [vector] or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the antibody-related light or

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Page 37, the last paragraph:

As used herein the term "rapamycin compound" includes the neutral tricyclic compound rapamycin, rapamycin derivatives, rapamycin analogs, and other macrolide compounds which are thought to have the same mechanism of action as rapamycin (e.g., inhibition of cytokine function). The language "rapamycin compounds" includes compounds with structural similarity to rapamycin, e.g., compounds with a similar macrocyclic structure, which have been modified to enhance their therapeutic effectiveness. Exemplary rapamycin [Rapamycin] compounds suitable for use in the invention, as well as other methods in which rapamycin [Rapamycin] has been administered are known in the art (See, e.g. WO 95/22972, WO 95/16691, WO

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Page 38, the third paragraph:

The antibodies of the invention are administered to subjects in a biologically compatible form suitable for pharmaceutical administration *in vivo* to inhibit immune responses. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the protein to be administered in which any toxic effects are outweighed by the therapeutic effects of the antibody. The term "subject" [subject] is intended to include living organisms in which an immune response can be elicited, e.g., mammals. Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. Administration of an antibody of the invention as described herein can be in any pharmacological form including a therapeutically active amount of anti-B7 antibody alone or in combination with an antibody reactive with another B lymphocyte antigen

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[(e.g., B7-1)] and a pharmaceutically acceptable carrier. Administration of a therapeutically active amount of the therapeutic compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of an anti-B7 antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of peptide to elicit a desired response in the individual. A dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

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Page 44, the fourth paragraph:

The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated herein by reference. Each reference disclosed herein is incorporated herein by reference [herein] in its entirety. Any patent application to which this application claims priority is also incorporated herein by reference [herein] in its entirety.

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Page 44, the last paragraph:

Intestine grafts were transplanted from B6C3F1/J mice into C57BL/6J wild-type or B7.1$^{-/-}$ B7.2$^{-/-}$ (B7$^{-/-}$) recipients. Wild-type recipients received either no treatment, mCTLA4Ig, or a combination of anti-B7-1 [anti-B7.1] and anti-B7-2 mAbs (for all 3 agents doses were 50 μg every other day at 7 doses). Rejection was graded histologically from 0 to 3 (no to

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Page 45, second paragraph:

Rejection scores of all syngeneic grafts were 0. As indicated by the mean rejection scores shown in Table 1, mCTLA4Ig had no effect on allograft rejection in wild-type mice. In contrast, blockade of the CD28/B7 pathway using anti-B7 mAbs

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significantly inhibited rejection ($p < 0.05$ at 28 days). The complete disruption of this pathway using B7 $^{-/-}$ recipients also resulted in a significant inhibition of rejection ($p < 0.001$). Examination of cytokine gene expression revealed that mCTLA4Ig had little or no effect on IL-2, IFNγ, α-TNF [aTNF], or IL-12 levels. In contrast, each of these cytokines was significantly decreased in anti-B7 mAb-treated or B7$^{-/-}$ recipients. Similarly, mCTLA4Ig-treated mice expressed levels of the chemokines RANTES and MIP-1 and their receptor CCR5 that were comparable to untreated recipients while anti-B7 mAb-treated and B7-/- recipients expressed decreased levels of these chemokines and CCR5.

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Table 1, of page 45:

Table 1: Mean Rejection Scores in Wild-Type Mice After Allogenic Transplant

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	Untreated	mCTLA4Ig	anti-B7-1 [anti-B7.1] and B7-2 [B7.2] mAB	B7.1$^{-/-}$/B7.2$^{-/-}$
Day 14	2.4±0.9	2.6±0.7	1.0±1.4	not done
Day 18/28	2.7±0.7	not done	1.3±0.5	0.8±0.4